METHOD FOR LOADING AND UNLOADING MACRO-MOLECULES FROM MICROFLUIDIC DEVICES

[0001] This application claims priority to provisional application Serial Number 60/442,309, filed October 30, 2002, which is incorporated by reference in its entirety.

Field of the Invention

[0002] The present invention relates to an integrated microfluidic device. More specifically, the present invention relates to methods and devices for loading or unloading a sample comprising charged molecules onto or out of an integrated microfluidic device.

Background

[0003] The separation of large biomolecules such as nucleic acids and proteins is a fundamental analytical and preparative technique in biology, medicine, chemistry, and industry. Recently, microfabricated structures exploiting various ideas for deoxyribonucleic acid (DNA) separation have been demonstrated. Han, J. & Craighead, H.G., *Science* **288**, 1026-1029 (2000); Turner, S.W., Cabodi, M., Craighead, H.G., *Phys Rev Lett.* 2002 Mar 25;88(12):128103; Huang, L.R., Tegenfeldt, J.O., Kraeft, J.J., Sturm, J.C., Austin, R.H. and Cox, E.C., *Nat Biotechnol.* 2002 Oct; 20(10): 1048-51; Huang, L.R., Silberzan, P., Tegenfeldt, J.O., Cox, E.C., Sturm, J.C., Austin, R.H. and Craighead, H. Role, *Phys. Rev. Lett.* **89**, 178301 (2002). Loading a DNA sample onto such a microfabricated devices is usually done by pipetting the DNA sample in an aqueous solution to a loading well (reservoir) (Figure 1). The DNA molecules are then transferred into the microfluidic channels using electric fields or hydrodynamic pressure.

[0004] Loading large DNA molecules (>200 kilo-base pair) onto microfluidic devices is however, more complicated, because large DNA molecules are susceptible to shearing and pipetting has to be avoided. Large DNA molecules may be embedded in

agarose gel plugs during sample preparation and manipulation to prevent shearing [Kurien, B.T. and Scofield, R.H. *Anal. Biochem.* **302**, 1-9 (2002)]. Loading DNA molecules from gel plugs onto microfluidic devices therefore requires the extraction of DNA fragments from gel plugs and loading DNA fragments on microfluidic devices without pipetting. Although many methods for extracting DNA fragments from gels have been developed, integrating these conventional methods with microfluidic devices remained challenging.

[0005] One standard method for extracting DNA molecules from agarose gel is electroelution, where DNA fragments are extracted out of the gel using electric fields (Figure 2). Electro-elution refers to a technique in which molecules embedded in a matrix, typically agarose gel, are extracted or recovered from the matrix using electric fields. For example, in electro-elution of DNA, a gel plug containing the DNA to be eluted is typically immersed in an electrophoresis buffer solution in a receptacle. Electric fields are applied to the buffer solution through a pair of immersed electrodes. Usually, direct current (DC) fields were sufficient to elute the DNA molecules from the gel plug [Kurien, B.T. and Scofield, R.H. *Anal. Biochem.* **302**, 1-9 (2002)], although it has been suggested that repeatedly inverted fields may be more effective for DNA molecules larger than 20,000 base pairs (20 kb) [United States Patent 4,959,133]. The eluted molecules are dissolved in the buffer solution.

[0006] Transferring the dissolved DNA molecules to microfluidic devices remained challenging—pipetting creates shear stress, which tend to break large DNA molecules (>50 kb) into pieces.

[0007] Analogous to loading DNA in solution (Figure 1), a possible method of integrating the electroelution technique with microfluidic devices is by adding a gel plug containing DNA in the loading well, as shown in Figure 3. Note that there is only one electrode for each reservoir/well. Because the total amount of electric current flowing from one electrode to the other is the same throughout the device, the current density in the loading well and the buffer reservoir is orders of magnitude smaller than that in the microfluidic channels. By Ohm's Law, the fields in the loading well are also negligibly small. This is because the cross-section of the loading well is orders of magnitude larger than

those of the microfluidic channels, and the electric fields in the loading well are virtually zero. Therefore, DNA fragments cannot be efficiently extracted from the gel plug. Further, the time required for the extracted molecules to reach the channels is prohibitively long. Thus, this method is, impractical for microfluidic devices because the fields in the loading well are too weak to extract DNA efficiently from the gel plug, and the time required for the extracted DNA fragments to move to the microfluidic channels is prohibitively long. There is need for a practical method for loading DNA fragments from gel plugs onto microfluidic devices.

Summary of the Invention

[0008] The present invention provides an integrated microfluidic device for analyzing or processing charged molecules comprising a sample chamber and a microfluidic channel, wherein the sample chamber contains two or more electrodes capable of generating electric fields in the sample chamber and the microfluidic channel comprises an inlet and an outlet. The electric field is configured to transfer the charged molecules to an area of the sample chamber. The sample chamber may occur at the inlet of microfluidic channel or it may occur at the outlet of the microfluidic channel. When the sample chamber occurs at the inlet of the microfluidic channel, the electric field is configured to transfer the charged molecules to the inlet of the microfluidic channel.

[0009] In one embodiment, the present invention provides an integrated microfluidic device comprising a sample chamber and a micro-fluidic channel, wherein the sample chamber comprises two electrodes capable of generating an electric field within the sample chamber and the microfluidic channel comprises an inlet and an outlet. The electric field is configured to transfer the charged molecules in a sample to the inlet of the microfluidic channel.

[0010] In another embodiment, the present invention provides an integrated microfluidic device comprising a sample chamber and a micro-fluidic channel. The microfluidic channel comprises an inlet and an outlet. The sample chamber occurs at the inlet of the microfluidic channel and contains two or more electrodes capable of generating

an electric field within the sample chamber and a section of matrix material comprising charged molecule. The electric field is configured to electro-elute the charged molecules from the section of matrix material and to transfer the charged molecules to the inlet of the microfluidic channel.

[0011] In another embodiment, the present invention provides an integrated microfluidic device comprising a sample chamber and a micro-fluidic channel. The microfluidic channel comprises an inlet and an outlet. The sample chamber occurs at the outlet of the microfluidic channel and contains two or more electrodes capable of generating an electric field in the sample chamber. The electric field is configured to transfer charged molecules from the outlet of the microfluidic channel into the sample chamber.

[0012] In another embodiment, the present invention provides an integrated microfluidic device comprising a sample chamber and a micro-fluidic channel. The microfluidic channel comprises an inlet and an outlet. The sample chamber occurs at the outlet of the microfluidic channel and contains a section of matrix material and two or more electrodes capable of generating an electric field in the sample chamber. The electric field is configured to transfer charged molecules from the outlet of the microfluidic channel into the section of matrix material.

[0013] In another embodiment, the present invention provides a method for loading charged molecules from a section of matrix material onto an integrated microfluidic device comprising a loading chamber and a micro-fluidic channel. The method comprises introducing the section of matrix material containing the charged molecules into the loading chamber, wherein the loading chamber comprises two electrodes, applying an electric field across the loading chamber; electro-eluding the charged molecules from the section of matrix material; and delivering the charged molecules to the microfluidic channel.

[0014] In another embodiment, the present invention provides a method for loading charged molecules onto a section of matrix material contained in an integrated microfluidic device. The integrated microfluidic device has an unloading chamber comprising two

electrodes and the section of matrix material, and a micro-fluidic channel comprising an inlet and an outlet. The method comprises applying an electric field across the unloading chamber; transferring the charged molecules from the outlet of microfluidic channel into the unloading chamber; and delivering the charged molecules onto the section of matrix material.

Brief Description of the Drawings

[0015] Figure 1 shows a cross-sectional diagram of a typical microfluidic device loaded with DNA fragments. DNA fragments in an aqueous buffer solution are pipetted to the loading well (reservoir), and injected into the device using electric fields.

[0016] Figure 2 shows a conventional apparatus for electro-elution of DNA. DNA fragments are extracted out of the gel plug immersed in an aqueous buffer solution using electric fields.

[0017] Figure 3 shows an impractical embodiment of integrating electroelution technique with microfluidic devices.

[0018] Figure 4 shows a cross-sectional diagram of an embodiment of the invention, showing a microfluidic channel integrated with a sample loading chamber comprising a gel plug immersed in aqueous buffer solution and two electrodes. Electrode 1 and 3 generate fields for extracting DNA fragments from the gel plug, whereas electrode 2 controls independently the fields across the microfluidic device.

[0019] Figure 5 shows a close-up diagram of the loading chamber in Figure 4, showing electric field lines generated by the two electrodes. Note that the arrows at the ends of the field lines indicate the direction of migration of DNA, not the electric field direction. Molecules move towards electrode 3.

[0020] Figure 6 shows a cross-sectional diagram of another embodiment of the disclosed method, showing a vertical loading chamber comprising two electrodes and a gel plug.

[0021] Figure 7 shows a cross-sectional diagram of another embodiment of the invention, showing a loading chamber comprising two loading wells connected by a fluidic channel, two electrodes and a gel plug.

[0022] Figure 8 shows a fluorescence micrograph of DNA fragments in micro-channels. The Lambda phage DNA multimers (50 kb – 1 Mb) is prepared in 1% agarose gel, extracted using the disclosed method at ~4V/cm for ~1 h, and injected in ~1 μm deep channels using ~5 V/cm. The scale bar is 20 μm.

[0023] Figure 9 shows a comparison of conventional loading technique to the disclosed method. (a) Conventional loading technique has a "dead volume" at the interface (inlet or outlet) between microfluidic channels and the loading well (sample chamber). Sample molecules have to move through the dead volume via diffusion, which is a very slow process, because the fields inside loading well are negligibly small. (b) Application of the disclosed method to loading of macro-molecules in aqueous solutions, showing that the fields penetrate the dead volume and deliver sample molecules to the (inlet of) microfluidic channel. Note that the arrows at the ends of the field lines indicate the direction of migration of DNA, not the electric field direction.

[0024] Figure 10 shows a cross-sectional diagram of the unloading well (sample chamber) showing two electrodes generating fields for recovering molecules from the microfluidic device. The sample molecules could be loaded to a gel plug if desired. Note that the arrows at the ends of the field lines indicate the direction of migration of DNA, not the electric field direction.

[0025] Figure 11 shows a cross-sectional diagram of a loading well (sample chamber) comprising two electrodes and a gel plug, showing that molecules are eluted from

the gel plug and transferred to the inlet of a microfluidic channel. Note that the arrows at the ends of the field lines indicate the direction of migration of DNA, not the electric field direction.

[0026] Figure 12 shows a cross-sectional diagram of the sample chamber for loading DNA within a gel plug onto a microfluidic channel. The gel plug is mounted on an electrode. . The arrows at the ends of the field lines indicate the direction of migration of DNA, not the electric field direction.

[0027] Figure 13 shows a schematic diagram of an integrated device of the present invention, comprising a microfluidic chip and a chip holder. (A) Schematic top view of the device, showing a sample chamber comprising two electrodes and a gel plug, and a fluidic reservoir. (B) Schematic cross-sectional side view of the device, showing the microfluidic chip connected to the chip holder with "o"-rings. (C) Schematic bottom view of the integrated device, showing the microfluidic chip comprising holes for fluid access and a microfluidic channel, mounted on the chip holder.

[0028] Figure 14 shows two fluorescence images of coliphage λ DNA multimers in a microfluidic channel.

[0029] Figure 15 shows a schematic cross-sectional diagram of the device of the present invention for DNA analysis, comprising a sample chamber comprising two electrodes and a gel plug, a fluid reservoir, and an array of microfluidic channels.

[0030] Figure 16 shows a schematic perspective diagram of the array of microfluidic channels shown in Figure 15. The coverslip is to be bonded to the substrate.

[0031] Figure 17 shows a fluorescence image of coliphage λ DNA multimers in an array microfluidic channel, each of which is 60 nm wide.

[0032] Figure 18 shows a schematic top-view of an integrated device comprising a sample chamber comprising three electrodes for generating fields of different directions.

Detailed Description

[0033] The method and device of the invention uses two or more electrodes in a sample chamber to create electric fields for electro-elution, sample transfer, or unloading. An integrated microfluidic device of the invention comprises a sample chamber and a microfluidic channel, wherein the sample chamber contains two or more electrodes capable of generating an electric field in the sample chamber and the microfluidic channel comprises an inlet and an outlet. In one embodiment of the invention, the sample chamber occurs at the inlet of the microfluidic channel. In this embodiment, the electric field in the sample chamber serves to deliver the charged molecules to the inlet of the microfluidic channel. In another embodiment of the invention, the sample chamber occurs at the outlet of the microfluidic channel. In this embodiment, the electric field in the sample chamber serves to transfer the charged molecules from the outlet of the microfluidic channel into the sample chamber.

[0034] In a preferred embodiment, the sample chamber occurs at the inlet of the microfluidic channel and comprises two electrodes and a section of matrix material containing the charged molecules. In this embodiment, the electric field in the sample chamber functions to electro-elute the charged molecules from the section of matrix material, and to transfer the charged molecules to the inlet of the microfluidic channel. In another preferred embodiment, the sample chamber comprising a section of matrix material occurs at the outlet of the microfluidic channel. In this embodiment, the electric field in the sample chamber functions to remove the charged molecules from the outlet of the microfluidic channel, transfer the charged molecules through the sample camber, and load the charged molecules into the section of matrix material. By loading the charged molecules onto the section of matrix material, the molecules are protected from shear stress and may be taken off the microfluidic device, for further analysis, processing or manipulation.

[0035] The present invention is useful for the manipulation of charged biological molecules including nucleic acids (*i.e.*, DNA, etc.), proteins and protein complexes as well as other nonbiological charged molecules, such as industrial polymers. The molecules may be inherently charged (for example, DNA) or may be chemically modified to carry a charge (for example, sodium dodecyl sulfate (SDS) treated protein). Thus, the term "charged molecules" as used herein refers to the charge-bearing biological or non-biological molecules or supra-molecules that are the subject of analysis or manipulation. In a preferred embodiment, the charged molecules are deoxyribonucleic acids, and in particularly preferred embodiments the charged molecules are deoxyribonucleic acids greater than about 50 kilo-bases in molecular weight. In another preferred embodiment, the charged molecules are proteins (polypeptides). The present invention provides a method for loading or unloading these molecules, which may be susceptible to shearing, onto or out of microfluidic devices, and may provide the advantages of high speed, low sample volume, and low manufacturing cost, etc.

[0036] The term "sample chamber" as used herein refers to a well, reservoir or cavity which contains a fluid medium. A sample chamber may comprise two or more compartments, inter-connected by one or more channels (for example, Figure 7). We refer to the inter-connected compartments as one single sample chamber if the fluid medium may flow freely between the compartments, and the compartments are well-connected electrically (fields in the compartment remain large enough for transporting or eluting the charged molecules). The sample chamber contains at least two electrodes that establish an electric field, or fields, in the chamber. The electric field may be direct-current (DC), alternating current (AC), or repeatedly inverted [*see* United States Patent 4,959,133, which is incorporated herein by reference in its entirety]. The electric field in the chamber functions to transfer charged molecules between the microfluidic channel and the chamber, which may comprise one or more sections of matrix material. The electrodes employed in the subject device may be any suitable type capable of producing an appropriate electric field to the medium in the chamber. Also, the electrodes employed in the subject device may be in any suitable shape to generate fields capable of electro-elution or transporting the molecules in the chamber.

[0037] The term "channel" as used herein refers to a structure wherein fluid may flow. A channel may be a capillary, a conduit, a strip of hydrophilic pattern on an otherwise hydrophobic surface wherein aqueous fluids are confined, etc. The term "microfluidic" as used herein, refers to a system or device having one or more fluidic channels, conduits or chambers that are generally fabricated at the millimeter to nanometer scale, e.g., typically having at least one cross-sectional dimension in the range of from about 30 nm to about 3 mm.

[0038] The term "integrated" as used herein refers to a device in which all of the components of the device (e.g., the chamber, electrodes, channels, etc.) are present as a single unit.

[0039] The term "matrix material" as used herein refers to any medium or solid support that may carry the charged molecules. The term matrix material includes, but is not limited to, gels such as agarose gel, polyacrylamide gel, etc., blotting materials such as nitrocellulose, nylon membranes, PVDF, etc., and solid supports such as a functionalized glass surface to which charged molecules can adsorb. The term section refers to a piece, plug or strip of the matrix material. In preferred embodiments the section of matrix material is a gel plug.

[0040] In a preferred embodiment of the invention, the sample chamber is a loading chamber, in which a sample comprising the charged molecules may be added. In this embodiment, the loading chamber contains two or more electrodes and occurs at the inlet of the microfluidic channel. The electrodes are positioned in the loading chamber such that the electric field generated by the electrodes delivers the charged molecules to the inlet of the microfluidic channel. Thus, this embodiment of the invention is useful for enhancing the loading of charged molecules in aqueous solutions (Figure 9). The directions of the field lines in the drawings point to the directions of motion of the charged molecules, and thus may be in the opposite directions to the actual field. To avoid bubbles in microfluidic channels, microfluidic devices are usually wetted with pure buffer solutions first, and then the samples are added. Therefore, there is usually a "dead volume" at the interface between

the loading well (chamber) and the microfluidic channels (Figure 9a). In the case where the field in the loading chamber is negligibly weak, injection of samples into the channels could be time-consuming and unreliable, depending on the volume of the "dead volume." Using two or more electrodes in the loading chamber, molecules can be delivered to the inlet of the microfluidic device (Figure 9b).

[0041] In another embodiment, the charged molecules are loaded into the loading chamber within a section of matrix material, preferably a gel plug. The charged molecules are eluted from the gel plug and directed to the inlet of the microfluidic channel using the electric field. The field may be direct-current (DC), alternating current (AC), or repeatedly inverted. In one embodiment, the gel plug may be mounted on an electrode in the sample chamber (Figure 12). In other embodiments, the gel plug may be placed between two electrodes in the sample chamber (Figure 5, 6, 7, 11). Preferably, the electrodes are positioned in the chamber such that the same electric field lines passing the inlet of the microfluidic channel go through the gel plug (see Figures 11 and 12).

[0042] In another embodiment, the charged molecules are loaded within a section of matrix material (for example, a gel plug) into the loading chamber comprising three electrodes (Figure 18). The three electrodes may generate electric fields of changing directions, known as cross fields, which may be more effective for eluting molecules from the a section of matrix material then DC or inverted fields. When voltages are applied to electrodes 1 and 2, field *E1* can be created; when voltages are applied to electrodes 1 and 3, field *E2* can be created. E1 and E2 are applied alternatively, so that the charged molecules are extracted from the section of matrix material.

[0043] The method and devices of the invention can be used for unloading samples from a micro-fluidic channel. In this embodiment the sample chamber, termed an "unloading chamber," occurs at the outlet of the microfluidic channel, and serves to collect the charged molecules after they are eluted from the microfluidic channel. The amount of sample coming out of a microfluidic device may be little and hard to recover with pipettes. Applying two or more electrodes in the unloading chamber (Figure 10) to generate electric fields enables a

sample of the charged molecules to be unloaded from the outlet of the microfluidic channel and to be collected in an area of the unloading chamber, for example, an area accessible by pipette tips. In one embodiment, the unloading chamber comprises a section of matrix material (e.g., a gel plug) in which the charged molecules are embedded. The section of matrix material may facilitate the transfer of charged molecules, especially those that are susceptible to shearing (e.g., DNA molecules larger than 50 kb), from the device.

[0044] In a preferred embodiment of the invention, the charged molecules are DNA molecules contained within a gel plug. The DNA molecules are extracted from the gel plug and loaded into the microfluidic channel using an electro-elution technique that employs two or more electrodes in a loading chamber to create strong electric fields for DNA extraction. One embodiment of this method is illustrated in Figure 4. Using two electrodes in the loading chamber, the electric fields across the gel plug can now be set to an optimum extraction strength, independent of fields inside the microfluidic channels. Depending on the application, the DNA fragments can be extracted first and then injected into the microfluidic device, or can be extracted as they are being injected.

[0045] The integration of the loading/unloading chamber with the microfluidic device may remove the need for pipetting. DNA molecules are transferred to the entrance (inlet) of microfluidic channels by electric fields (Figure 5). Because electric fields spread throughout the loading chamber, molecules are delivered to every part of the chamber towards the anode. The exact positions of the electrodes and the geometry of the loading chamber may not be critical, and thus other embodiments are also useful (for example, Figures 6 and 7). For example, the loading chamber could be vertical, with one electrode on top of the other (Figure 6), or could consist of more than one wells (chambers), separated by fluidic channels (Figure 7).

[0046] In a preferred embodiment of the invention, the device is microfabricated. Microfabrication techniques may be selected from those known in the art, for example, techniques conventionally used for silicon-based integrated circuit fabrication, embossing, casting, injection molding, and so on [E. W. Becker *et. al.*, Microelectronic Engineering 4

(1986), pages 35 to 56]. Examples of suitable fabrication techniques include photolithography, electron beam lithography, imprint lithography, reactive ion etching, wet etch, laser ablation, embossing, casting, injection molding, and other techniques [H. Becker *et. al.*, J. Micromech. Microeng. 8 (1998), pages 24 to 28]. The microfluidic device may be fabricated from materials that are compatible with the conditions present in the particular application of interest. Such conditions include, but are not limited to, pH, temperature, application of organic solvents, ionic strength, pressure, application of electric fields, surface charge, sticking properties, surface treatment, surface functionalization, and bio-compatibility. The materials of the device are also chosen for their optical properties, mechanical properties, and for their inertness to components of the application to be carried out in the device. Such materials include, but are not limited to, glass, fused silica, silicone rubber, silicon, ceramics, and polymeric substrates, e.g., plastics, depending on the intended application.

[0047] The microfluidic channel may be any kind known in the art for the analysis, manipulation, processing or separation of charged molecules. In one embodiment of the invention, the loading chamber is connected to a microfluidic channel connecting to a sieving matrix for DNA separation according to size [Kim, Y. & Morris, M. D. Rapid Pulsed Field Capillary Electrophoretic Separation of Megabase Nucleic Acids. *Anal. Chem.* **67**, 784-786 (1995).]. The sieving matrix may be microfabricated [Han, J. & Craighead, H.G. Separation of long DNA molecules in a microfabricated entropic trap array. *Science* **288**, 1026-1029 (2000); Turner, S.W., Cabodi, M., Craighead, H.G. Confinement-induced entropic recoil of single DNA molecules in a nanofluidic structure. *Phys Rev Lett.* 2002 Mar 25; 88(12):128103; Huang, L.R., Tegenfeldt, J.O., Kraeft, J.J., Sturm, J.C., Austin, R.H. and Cox, E.C. A DNA prism for high-speed continuous fractionation of large DNA molecules. *Nat Biotechnol.* 2002 Oct; 20(10):1048-51; and Huang, L.R., Silberzan, P., Tegenfeldt, J.O., Cox, E.C., Sturm, J.C., Austin, R.H. and Craighead, H. Role of molecular size in ratchet fractionation. *Phys. Rev. Lett.* **89**, 178301 (2002)]. Integrating the microfluidic channels with loading chambers comprising two or more electrodes makes the microfluidic channels more efficient, because "dead volumes" can be eliminated. Further, integrating the microfluidic channels with loading chambers comprising a section of matrix material (e.g., a gel plug) and

two or more electrodes expands the range of molecules the microfluidic channels can process, especially to molecules susceptible to shearing.

[0048] In another embodiment of the invention, the loading chamber is connected to a microfluidic channel, which connects to thin (~100 nm) microfluidic channels, for confining DNA molecules. The DNA molecules migrating through the thin channels are stretched to a linear conformation, which allows for the characterization of DNA according to size, the observation of protein-binding sites on DNA, the study of polymer dynamics, etc. [H. Cao, Z. N. Yu, J. Wang, J. O. Tegenfeldt, R. H. Austin, E. Chen, W. Wu, S. Y. Chou, *"Fabrication of 10 nm Enclosed Nanofluidic Channels," Applied Physics Letters* **81**, 174 (2002); J. O. Tegenfeldt, O. Bakajin, C. F. Chou, S. S. Chan, R. Austin, W. Fann, L. Liou, E. Chan, T. Duke, E. C. Cox, *"Near-Field Scanner for Moving Molecules," Physical Review Letters* **86(7)**, 1378 (2001)].

[0049] The electric field strength employed in the sample chamber is typically in the range of about 1 V/cm to about 30 V/cm. For highly conductive buffers, lower field strengths may be preferred, in order to avoid excessive bubble generation. When the electric field is employed to manipulate (*e.g.*, extract from a gel plug, embed into a gel plug, or transfer between sample chambers and microfluidic channels) molecules susceptible to shearing, field strengths used to perform pulsed-field gel electrophoresis (PFGE) [R. L. Miesfeld, *Applied Molecular Genetics* (Wiley, New York, NY, 1999), page 95-97] are preferred. Typically, the field strength is about 1 V/cm to about 10 V/cm, with lower strength for larger molecules. When repeatedly inverted fields (pulsed fields) are used, the durations of the electric pulses are typically between 1 sec and 5 min, depending on the molecular weights of the molecules being eluted.

[0050] It is understood that the various embodiments described herein are by way of example only, and are not intended to limit the scope of the invention. For example, many of the materials and structures described herein may be substituted with other materials and structures without deviating from the spirit of the invention. It is understood that various theories as to why the invention works are not intended to be limiting.

Examples

[0051] Specific representative embodiments of the invention will now be described, including how such embodiments may be made. It is understood that the specific methods, materials, conditions, process parameters, apparatus and the like do not necessarily limit the scope of the invention.

Example 1

[0052] Figure 13 shows schematically an integrated microfluidic device for manipulating genomic-sized DNA molecules comprising a microfluidic chip and a chip holder. The microfluidic chip is about 20 mm x 45 mm x 1 mm in dimension, and comprises two holes for fluid access to a microfluidic channel of 500 μm wide, 35 mm long and 100 μm deep. The microfluidic channel is fabricated on a silicon wafer using photolithography and deep reactive ion etching, techniques conventionally used for silicon-based integrated circuit fabrication. Holes through wafers for fluid access were made by sand-blasting. The silicon wafer is diced and sealed with a glass coverslip coated with silicone rubber (RTV-615 from General Electric). The glass coverslip allows for optical observation of molecules inside the microfluidic channel.

[0053] The chip holder has a sample chamber with two electrodes and a fluid reservoir having one electrode. The sample chamber is about 5 mm (width) X 50 mm (length) X 1 mm (height). The chip holder and the microfluidic chip are clamped together, and "o"-rings are used to form air-tight contacts. The sample chamber, the microfluidic channel, and the fluid reservoir are then filled with ~500 μL of an aqueous buffer containing 0.5 X Tris-Borate-EDTA, 0.1% POP-6, a performance optimized linear polyacrylamide (Perkin-Elmer Biosystems) and 10 mM dithiothreitol (DTT), added to suppress electroosmotic flow and photo-bleaching, respectively.

[0054] An agarose gel plug containing coliphage λ DNA multimers (50 μg/ml, 50 kb to 1 Mb) is placed in the sample chamber between one electrode and the inlet of the microfluidic channel (Figure 13). A direct-current (DC) voltage of –5 V is applied to the

electrode 1 for ~105 min, as electrode 2 and electrodes 3 are grounded. The direction of the field is such that the DNA molecules migrate towards the inlet of the microfluidic channel. After applying the field in the sample chamber (electro-elution), ~1 μL of a fluorescent dye (TOTO-1 from Molecular Probes, Eugene, OR) of ~3 μM were added at the inlet of the microfluidic channel 4 times using a pipette. The fluorescent dye stains the DNA molecules in the fluid. Staining of molecules may also be done by mixing the dye with the molecules, in principle, on the microfluidics chip part of the device.

[0055] The voltage on electrode 3 is then switched to +20 V, which creates a field of ~6 V/cm across the microfluidic channel. DNA molecules are driven through the channel by the electric field. Figure 14 shows fluorescence images of DNA molecules of 50 kb to 1000 kb in the channel. The method successfully elutes genomic-sized DNA molecules out of the gel plug, and transfers the molecules into the microfluidic channel.

Example 2

[0056] Figure 15 shows schematically an integrated microfluidic device for confining DNA molecules, comprising a sample chamber with two electrodes, a buffer reservoir and an array of parallel microfluidic channels of 60 nm x 60 nm cross-section. The array of microfluidic channels is fabricated on a fused silica substrate (Figure 16), on which 60 nm wide channel patterns are defined using nano-imprint lithography [S. Y. Chou, *et. al.*, *"Imprint Lithography with 25-Nanometer Resolution," Science* **272**, 85 (1996).]. The exposed substrate surface is etched by 60 nm using reactive-ion-etching. After cleaning, the substrate is bonded with a fused-silica coverslip, where holes for fluid access are made. The sample chamber and the fluid reservoir are made of cut pipette tips adhesively attached to the coverslip.

[0057] The sample chamber, the microfluidic channel, and the fluid reservoir are then filled with an aqueous buffer containing 0.5 X Tris-Borate-EDTA, 0.1% POP-6, a performance optimized linear polyacrylamide (Perkin-Elmer Biosystems) and 10 mM dithiothreitol (DTT). An agarose gel plug containing coliphage λ DNA multimers (50 μg/ml, 50 kb to 1 Mb) is mounted on electrode 1 and immersed into the aqueous buffer in the

sample chamber. The electric pulses of –6 V for 30 sec and +3V for 10 sec are applied repeatedly for ~2 hr to electrode 1, as electrode 2 and 3 are grounded, to create pulsed fields in the sample chamber for electro-elution of the DNA. The net migration of the DNA molecules is from the gel plug to the inlet of the microfluidic channel. After electro-elution, ~1 μL of a fluorescent dye (TOTO-1 from Molecular Probes, Eugene, OR) of ~3 μM were added at the inlet of the microfluidic channel 4 times using a pipette. The fluorescent dye stains the DNA molecules in the fluid.

[0058] The voltage on electrode 3 is then switched to +10 V, in order to create a field of ~4 V/cm across the microfluidic channel. Consequently, the DNA molecules of 50 kb to 1000 kb are driven to into the small channels (Figure 17). Because of the confinement of the DNA by the microfluidic channels, the DNA molecules are linearly stretched. The molecules can then be studied using an optical microscope.

[0059] This device may be used to identify the binding sites of DNA binding proteins, including transcription factors, on the DNA molecules. Genomic-sized DNA samples binding to transcription factors can be isolated from biological cells embedded in a gel plug. The purified DNA samples can then be loaded into the device, where the DNA molecules are stretched [J. O. Tegenfeldt, O. Bakajin, C. F. Chou, S. S. Chan, R. Austin, W. Fann, L. Liou, E. Chan, T. Duke, E. C. Cox, *"Near-Field Scanner for Moving Molecules,"* *Physical Review Letters* **86(7)**, 1378 (2001)].

[0060] While the present invention is described with respect to particular examples and preferred embodiments, it is understood that the present invention is not limited to these examples and embodiments. The present invention as claimed therefore includes variations from the particular examples and preferred embodiments described herein, as will be apparent to one of skill in the art.